Liquid-assisted Mechanochemical Synthesis: Green Approach to Synthesis of Co(II) Schiff Base Complex and of Evaluation of Antimicrobial Activities

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Abstract:
The use of liquid-assisted grinding to bring about reaction between solids, can offer lower-west paths to chemical product by avoiding bulk solvent during reaction. The schiff base ligand derived from 2-hydroxy-1-napthaldehyde and 1,2-phenylenediamine namely 2,2'-[1,2-Phenylenebis(nitrilomethylidyne)] bis-naphthol; $[H_2(L)]$ and its corresponding Co(II) complex were synthesized using liquid assisted mechanochemical grinding method and characterized by means of IR spectroscopy, powder X-ray diffraction, differential scanning calorimetry, Thermogravitric analysis, energy dispersive X-ray and CHN analysis. The schiff base have been found by a conventional solution method for comparison. In the complex compound, the coordination environment around metal centre consist of two Nitrogen and two oxygen atoms of the schiff base ligand which forms square planar geometry. Evidence from Infrared spectral study indicated a strong band in the spectra of free ligand occurring at $1641cm^{-1}$ which assigned to $\nu(C=N)$. It shifted to lower frequency region $1625cm^{-1}$ in the complex indicating the involvement of nitrogen atom of azomethine group. The coordination of Schiff base with the metal ion as showed by the appearance of low frequency band at $452cm^{-1}$ due to metal-oxygen $\nu(M-O)$ vibration thus confirming participation of oxygen in the coordination. Further coordination of metal to azomethine nitrogen $\nu(M-N)$ is confirmed by a new band at $753cm^{-1}$. The PXRD reveals that, the patterns of the ground mixture were entirely different from the starting constituents, indicating that all the starting materials were transformed to product furthermore the melting point/decomposition temperature and colour of the complexes were different from that of the schiff base indicating the formation of coordination compounds. The synthesized schiff base ligand, and metal complex were screened for its antibacterial activity using gram positive (Staphylococcus aurous) and gram negative (Escherichia coli) and antifungal activity using Aspergillum flavus. (gram positive)and Aspergillus niger. (gram negative)as potential antimicrobials and showed moderate activity.

Keywords: Mechanochemistry, Liquid-assisted Grinding, schiff base, Antimicrobial activity

1. Introduction

It has been claimed recently that, while traditional synthesis approaches have been incredibly successful, they are innately wasteful, and as raw materials become more limited, it is essential that we struggle to make synthetic chemistry more efficient (Mason*et. al.*, 2007). The traditional synthesis methods mentioned here are solution-based batch processes, and the inefficiencies related with them arise mainly from the need to use large amounts of solvent in the reaction, with the attendant need for separation of products by chromatography, crystallization, etc. The above remark was made in relation to proposals to substitute batch solution-based processes with solid-state synthesis methods. Mechanochemical synthesis involves a reaction between ''dry'' reactants, i.e. with no added liquid that might act as a solvent. However, some reactions in this type can result in the generation of liquid during the reaction, e.g. when any one of the reactants is a hydrate, producing liquid water during the reaction, or when liquid by-products such as water or acetic acid are produced as condensates during the reaction (Friscic 2010; Friscic 2012; Chieng *et. al.*, 2011; Bowmaker *et. al.*, 2008). These types of reactions are detected to proceed rapidly under mechanochemical conditions, signifying that the liquid produced during the reaction may accelerate it. For example, is the studies of mechanochemical reactions that can occur when milling and pressing analytes with alkali halides to form disks for IR experiments have shown that these reactions are accelerated when the analyte is a hydrate (Fernandez-Bertran, 1997). In contrast to this is the other class of mechanochemical reactions in which a very small quantity of liquid is intentionally added to the reaction mixture. In literature, the first report of such a process involved a study of

mechanochemical co-crystal formation (formation of crystalline molecular adducts from solid organic reactants) in the presence of little amounts of ''appropriate'' solvents, indicating a significant improvement in the rate of product formation in the presence of such solvents (shan, *et. al.,* 2002). Subsequent studies have revealed that very small quantity of added liquid can dramatically accelerate, and even allow, mechanochemical reactions between solids. Usually the molar equivalents added are comparable to those of the reactants themselves, and are orders of magnitude smaller than quantities used in solution-based reactions. Such reactions are hence more properly described as ''minimal solvent'' rather than strictly ''solvent-free'' (James *et al.*, 2012). They are much more related closely to typical solid-state mechanochemical reactions than to solution-based reactions. sometimes mechanochemical reaction produces by-products that need to be removed, or if there is need to grow larger crystals of the product (e.g. for X-ray crystallography), then conventional solution methods such as washing and recrystallization become involved. Mechanochemical method of synthesis is a topic of increasing interest to synthetic chemists (James *et. al.,* 2012). Particularly relevant entitled "The synthesis mind set" states: "The usual question when planning synthesis is still 'which solvent should I use?' rather to think 'do I need a solvent?' For broader acceptance of mechanochemistry, change in this respect is needed and this aspect may be as significant as overcoming technical obstacles." This is a very important point, and a way of bringing about such a "wider acceptance" is to show the significance of mechanochemical method relative to the conventional solution methods for example the yield of product from mechanochemical syntheses is usually higher than that of conventional solution syntheses, and in many cases, is essentially quantitative (James *et. al.,* 2012; Friscic, and Jones 2009).

In this work, we have investigated the solvent-assisted mechanochemical synthesis of a widely-used class of organic imine-based ligand. Specifically, diamine-type ligand which are formed from 2-hydroxy-1-naphthaldehyde and 1,2-phenylenediamine under solvent assisted mechanochemical conditions. We then investigated metal complexation using Co(II) acetate as test for two step mechanochemical syntheses. Diamine-type schiff base complexes are of wide interest, example for fluorescent indi-cators (Germain, and Knapp, 2008). biological mimics (Jones, *et. al.,*1979), 'Jacobsen' epoxidation catalysts (Jacobsen, *et. al.,* 1991). extraction of metal ions from water(In, *et. al.,* 2008), ring-opening poly-merisation catalysts (Idage *et. al.,* 2010) and organic light emitting diode (OLED) applications (Bhattacharjee, *et. al.,* 2011) Therefore, low-waste routes to such materials are of practical as well as academic interest.

2. Material and Method
Grinding in all the reactions was carried out in agate mortar with pestle. Chemical used in this study were obtained from Sigma Aldrich UK in >95% purity. All materials were used as obtained without further purification.

2.1. Instrumentation
Solid state IR spectra were recorded on a Perkin-Elmer FTIR Spectrum-400. PXRD measurements were carried out on a PAN analytical EmpyreanX'Pert Pro X-ray diffractometer. Copper was used as the X-ray source with a wavelength of 1.5405 Å. All experiments were carried out ex-situ using a spinning stage. Diffractograms were typically carried out from 5–40° with a step size of 0.0167°. Energy dispersive X-ray (EDX) were determined using FESEM/EDX Hitachi brand model SU8220. DSC were carried out using TA DCS Q20 V24.10 instrument with cooling accessories of -180 to 350°C. Thermal analysis studies of complexes (TGA) were performed by Perkin-Elmer Pyris Diamond TG/ DTA Heated in flowing Nitrogen (200mlmin^{-1}). Elemental microanalysis of separated solid chelates for C, H and N were determined using a Perkin-Elmer CHNS/O 2400 series II microanalyse.

2.2. Mechanochemical synthesis of Schiff base

2.2.1. 2,2'-[1,2-Phenylenebis(nitrilomethylidyne)] bis-naphthol; [H$_2$(L)] Schiff base
2-hydroxy-1-naphthaldehyde, 2mmol (0.3444g) and 1,2-phenylenediamine 1mmol (0.1081g) were weighed in to agate motor, a small amount of DMF (2-3 drops i.e. 0.1-0.2ml) was added and the mixture was ground for 30min to obtained light brown powder. The solvent was removed from the resulting paste of product by allowing it to stand for some time in the air.

2.3. Mechanochemical synthesis of Complex

2.3.1. 2,2'-[1,2-Phenylenebis(nitrilomethylidyne)]-bis[naphthalato]-N, N',O, O'-Cobalt(II); [Co(L)] Complex
[H$_2$(L)]schiff base (1mmol, 0.4164g) and Cobalt acetate tetrahydrate (1mmol, 0.2491g) were weighed in to agate motor, a small amount of DMF (2-3 drops i.e. 0.1-0.2ml) was added and the mixture was ground for 30min to obtained dove powder. The solvent was removed from the resulting paste of product by allowing it to stand for 12h in the air.

2.4. Solution based Synthesis of Schiff bases
Solution based reaction were carried out in a 250ml necked round bottomed flask equipped with a magnetic stirrer and reflux condenser. Products were obtained by filtration and dried in a desiccator over silica crystals for 36 hours.

2.4.1. 2,2'-[1,2-Phenylenebis(nitrilomethylidyne)] bis-naphthol; [H₂(L)] Schiff base

The Schiff base ligand [H₂L] was prepared as described by Raman *et al.,* 2004. This was done by the condensation of 25ml of 2-hydroxy-1-naphthaldehyde (1.722, 10mmol) with 1,2-Phenylenediamine (0.5405g, 5mmol) in ethanol (2:1 molar ratio). The mixture was then refluxed for 3h. The product obtained was filtered, washed with ethanol, dried, and preserved in a desiccator containing CaCl₂.

2.5. Microbial Activity

The antimicrobial activities of the schiff base and its Co(II) complex in dimethylsulfoxide (DMSO) were performed *in vitro* by double dilution method. The schiff base and the complex were dissolved separately in dimethylsulfoxide to produce three different concentrations (60, 30 and 15µgml^{-1}), which were placed on the surface of the culture and incubated at 37ºC for two days. The diameter of the zone of inhibition produced by the schiff base and complex were compared with that of the referenced drugs (Ciproflaxacin 500mg) for bacteria standard and (Ketoconozole 200mg) for fungal standard (Ramon *et al.,* 2003; Yeamin *et al.,* 2003).

3. Results and Discussion

The results of the analysis of the schiff base ligands and metal (II) complex are presented below:

	Molecular formula	Colour	Yeild (%)	Found (Calculated) (%)		
				C	H	N
[H₂(L)]	C₂₈H₂₀N₂O₂	Light brown	88.6	81.13(80.75)	4.39(4.84)	6.44(6.73)
[Co(L)]	C₂₈H₁₈CoN₂O₂	Dove	93.2	70.92(71.04)	3.58(3.83)	6.13(5.92)

Table 1: Physical properties of the schiff base ligand and its metal(II) complex

Compound	ν(C=N)	ν(O–H)	ν(C–H)	ν(C–O)	ν(C–C)	ν(M–N)	ν(M–O)
[H₂L]	1641	3396	3035	1215	1466	-	-
[Co(L)]	1625	-	2893	1242	1487	756	438

Table 2: Infrared spectral data of [H₂L] schiff base ligands and its metal complexes (cm^{-1})

Compound	Element	Weight (%)	Atomic (%)	K Ratio	Line type
[Co(L)]	C	62.20	68.97	0.16190	K Series
	N	11.09	10.54	0.00398	K Series
	O	23.83	19.84	0.01375	K Series
	Co	2.88	0.65	0.00854	K Series

Table 3: Energy dispersive x-ray (EDX) of Co(II) Complex

Compound	Hydration tempt (ºC)	Weight loss: observed (calculated)	H₂O of Hydration	Melting point/Decompositi on tempt. (ºC)
[H₂(L)]	-	-	-	126.3
[Co(L)]	152.0	1.40(1.22)	$^1/_3$	153.0

Table 4: Thermal analysis of schiff base and its Complex

Compound	Escherichia coli: zone of inhibition (mm)			Staphylococcus aureus: zone of inhibition (mm)		
	60µgml^{-1}	30µgml^{-1}	15µgml^{-1}	60µgml^{-1}	30µgml^{-1}	15µgml^{-1}
Schiff base	11	9	-	14	12	10
[Co(L)]	18	15	12	21	18	17

Table 5: Antibacterial activities of investigated schiff base and its Complex

Compound	Aspergillus flavus: zone of inhibition (mm)			Aspergillus niger: zone of inhibition (mm)		
	60µgml^{-1}	30µgml^{-1}	15µgml^{-1}	60µgml-1	30µgml^{-1}	15µgml^{-1}
Schiff base	11	8	-	12	-	-
[Co(L)]	24	21	18	16	15	13

Table 6: Antifungal activities of investigated schiff base and its complex

4. Discussion

Grinding of 2-hydroxy-1-naphthaldehyde with 1,2-phenylenediamine in a stoichiometric ratio did not result in a complete reaction, but partial reaction was observed, as evidenced by PXRD. Guided by the observation that small amounts of a liquid phase can significantly enhance the scope and rate of mechanochemical synthesis we turned to LAG (Dominik and Branko 2011; Kaitnerand Zbačnik 2012; Cinčić*et. al.,* 2011) Addition of a small quantity of dimethylsulphuroxide (DMF) to the reaction mixtures, followed by grinding for 30 min, quantitatively provided new materials that were characterised by FT-IR spectroscopy, PXRD, DSC, TGA, EDX, and CHN analysis as described below.

The schiff base ligand has a light brown colour with 88.6% yield and the elemental analysis results indicate that the experimental result is very closed to the calculated data as showed in table 1.The FT-IR spectra provide valuable information regarding the nature of functional group attached to the metal atom (Table 2). In order to study the bonding mode of schiff base to the metal complexes, the IR spectrum of the free ligand is compared with the spectra of the complexes. Preliminary identification of ligand was obtained from the absence of the IR bands characteristic for the amino and carbonyl groups and this is further confirmed by the appearance of new bands due to -C=N- of azomethine. The strong band observed at 1641 cm^{-1} in the spectra of schiff base can be assigned to C=N stretching vibration. The azomethine stretching frequencies in the spectrum of complex is shifted to lower frequencies when compared to free ligand. This indicates the coordination of metal center with azomethine nitrogen group. (Al-kubais*et. al.,* 1994; Selwin *et. al.,* 2006). It is expected that coordination of nitrogen to the metal atom would reduce the electron density in the azomethine link and thus lower the -HC=N absorption. The spectrum of schiff base [H$_2$(L)] also shows several bands corresponding to aromatic C-H stretching at 3035cm^{-1} and aromatic C-C stretching at 1466cm^{-1}. The spectra of schiff base ligand exhibit strong peak at 3396cm^{-1} which can be assigned due to O-H stretching vibration. The phenolic C-O stretching frequency of schiff base is seen at 1242cm^{-1}(Vadivela, and Dhamodaranba 2015). The O-H broadband in the Schiff base was no longer found in the spectra of investigated metal complex and also phenolic C-O stretching frequency get shifted in the complex indicating deprotonation and coordination of the hydroxyl oxygen to the metal ion. (Reddy *et. al.,* 2006).The new bands which are not present in the spectrum of schiff base ligand appeared at 756cm^{-1} corresponding to M-N and 438cm^{-1} assigned to M-O vibrations (Nakamoto, 1997). The appearance of M-N and M-O vibration support the involvement of N and O atoms in the complexation with metal ion under investigation (Jain *et. al.,* 2005). Therefore, the IR spectral data indicate that the coordination sites of the schiff base to metal ion are -C=N and -C-O. The IR spectra of schiff base and complex are depicted in figures Fig. 1
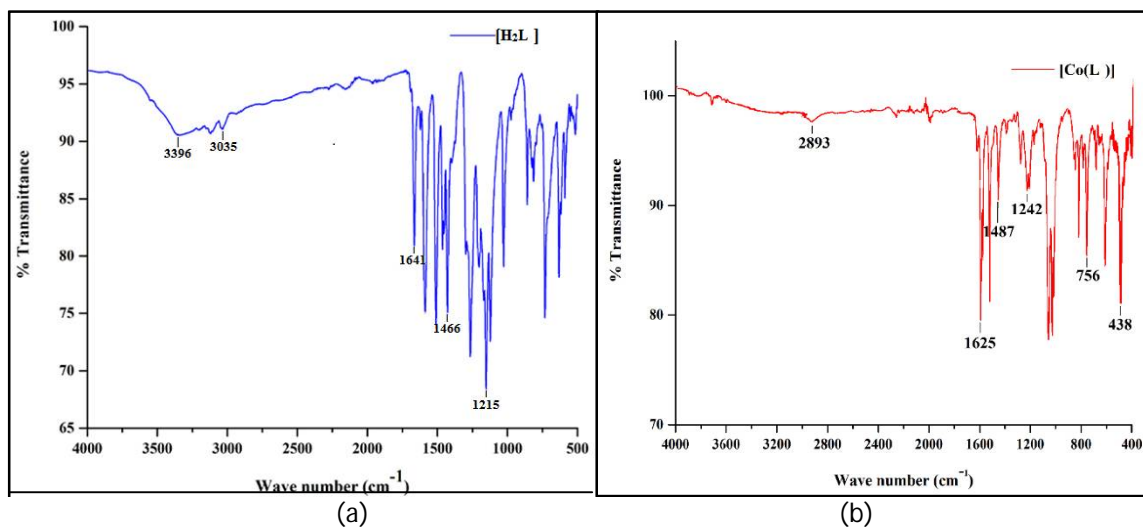

Figure 1: FT-IR spectrum of [H$_2$L^2] Schiff base (a) and [Co(L^2)]Complex (b) obtained mechanochemically

The X-ray diffraction patterns of the synthesized schiff base and its metal complexes revels the crystalline or amorphous nature of the compounds. The powder X-ray diffraction pattern of the schiff base and complex are scanned in the range of 5-40⁰C (θ), the diffractogram and associated data depict the 2θ value for each peak relative intensity and inter-planar spacing (d-value). The patterns of all the ground mixtures were completely different from the starting materials. Furthermore, no peak derived from the starting materials were observed, indicating that all starting materials were converted to product. The sharp reflections in the patterns testified to the crystallinity of the LAG products. The diffractogram of [H$_2$(L)] schiff base shows 25 reflections peaks with maxima at 2θ=7.590⬜.The diffractogram of [Co(L)]complex shows 17 reflections peaks with 2θ maxima at 25.27⬜. Fig. 2. and 3
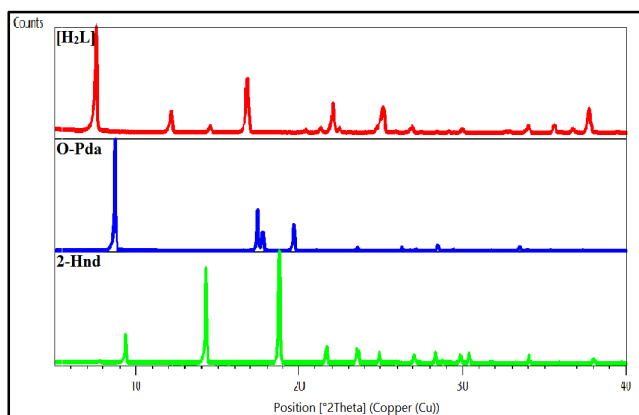
Figure 2: PXRD Patterns of pure 2-Hydroxy-1-naphthaldehyde (2-Hnd), pure 1,2-phenylenediamine (O-Pda) and [H₂L] schiff base
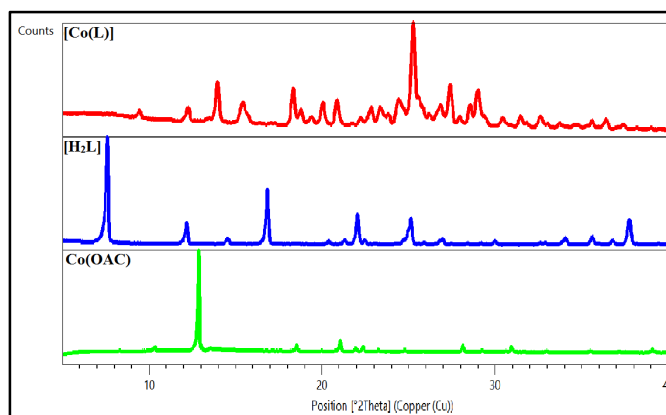

Figure 3: PXRD Patterns of Cobalt (II) acetate tetrahydrate (Co(OAC)), [H₂L] schiff base and [Co(L)] complex product after 30min LAG of Cobalt (II) acetate and [H₂L] schiff base

Energy Dispersive X-ray referred to as EDX (Table 3) is an X-ray technique used to identify the elemental composition of materials or chemical characterization of a samples. Its relies on the interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum. EDX allows the elemental composition of a sample to be measured. EDX can be used to determine which chemical elements present in the sample, and can be used to estimate their relative abundance. The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the samples being analyzed. Elemental mapping of a sample and image analysis are also possible as showed in fig. 4. EDX results showed that, Carbon has the largest atomic percent 68.97%, followed by oxygen 19.84% then Nitrogen 10.54% and Cobalt 0.65%. the atomic percent of all the four component elements were compared in all the three-point analyzed and the result were found to be in agreement with each other indicating the uniform distribution of all the component elements in the sample compound. Furthermore, the result is in conformity with the proposed structure of the complex.
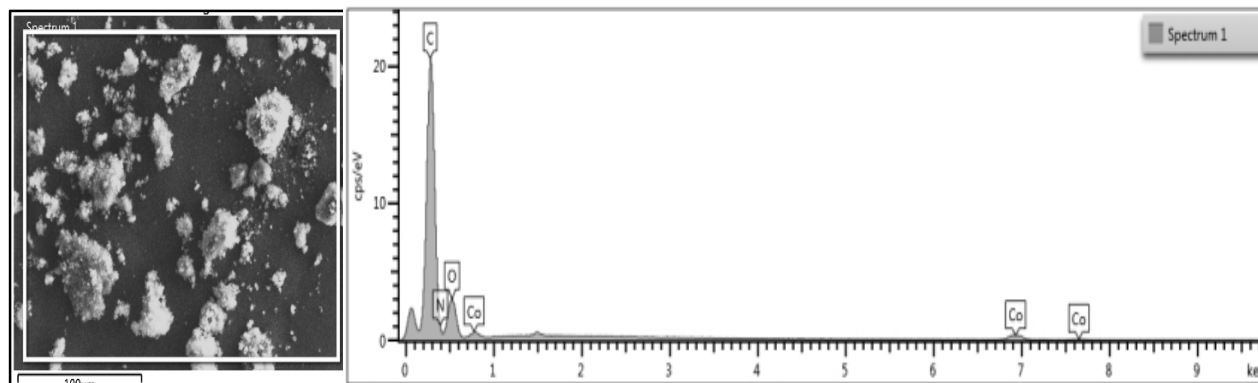

Figure 4: EDX analysis of [Co(L²)]complex showing peak due to Carbon, Nitrogen, Oxygen and Cobalt in the complex compound

The results for the thermal analysis of the schiff base and its complex are summarized in the table 4. Differential scanning calorimetry (DSC) curve of [H₂(L)] schiff base shows one endothermic peak at 126.3°C which corresponds to the melting point and decomposition temperature of the schiff base (fig. 5).The complex product was obtained as powder compound containing some amounts of residual water by-product as shown by TGA (Thermogravimetric analysis). However, on heating (105°C, 36h) complete loss of the water occurred to give a material with satisfactory elemental analysis. The TGA curve of the complex, shows 1.4% weight loss at 152°C which is equivalent to 1/3 molecule of water per molecule of complex, it's conformed to the calculated result 1.22%. Decomposition starts at 153°C and complete at 358°C (weight loss 34%) Fig. 6.
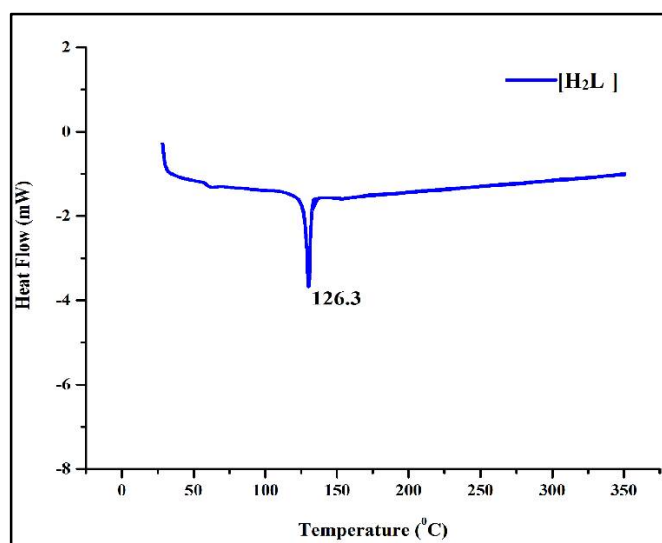
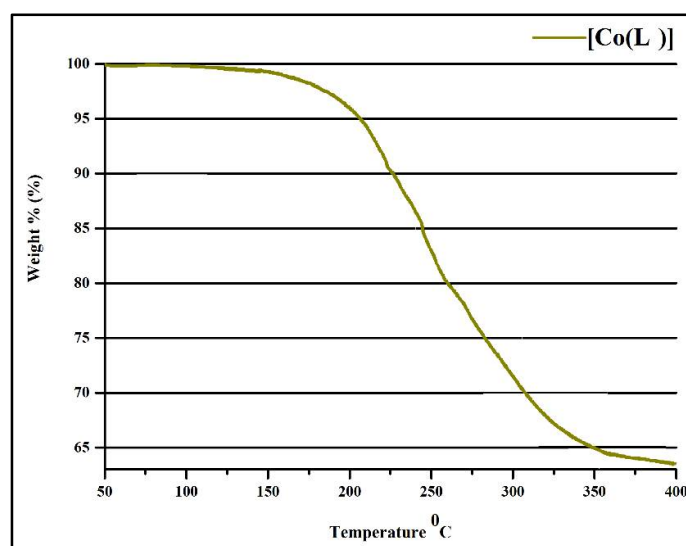*Figure 5: DSC curve of [H$_2$L] schiff base*

Figure 6: Thermogravimetric analysis of [Co(L)] Complex, obtained mechanochemically showing
1.4% weight loss at 152^{0}C which is equivalent to 1/3 molecule of H$_2$O per molecule of complex

The synthesized Schiff base and the complex showed moderate to good microbial activity against the organisms tested. In case of bacterial strains *Staphylococcus aureus* show highest activity in both schiff base and complex (14mm and 21mm respectively) at 60µgml^{-1} against *Escherichia coli* 11mm in the schiff base and 18mm in the complex (Table 5), the activities of both the schiff base and complex were compared to the reference drug Ciproflaxacin 500mg (45mm zone of inhibition), in both the two bacterial isolates tested Co(II) complex showed higher activity than the schiff base this is probably due to the greater lipophilic nature of complexes. Such increased activity of the metal chelates can be father explained on the basis of Overtone's concept and Tweedy's chelation theory (Tweedy 1964). On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor group (Kralova *et al.,* 2000, Parekh *et al.,* 2005) further, it increases the delocalization of π electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membrane and those block the metal binding sites on enzymes of microorganisms (Vaghasia *et al.,*2004).

Antifungal strains, *Aspergillus niger* has zone of inhibition of 11mm and 9mm at 60 and 30µgml^{-1}with the metal complex having higher zone of inhibition more than the derived Schiff base (Table 6). In case of *Apergillus flavus*, the effective antifungal activity was not observed against the schiff base, whereas the complex showed moderate activity compared to the reference drug Ketoconozole 200mg (32mm zone of inhibition). Thus, it can be said that, complexation or chelation increases antimicrobial activity as explained by Overton's concept and Tweedy's chelation theory. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group with the active centers of the cell constituents, resulting in an interference with normal cell process (Dharamraj *et al.,*2001).

From the analyses of the Schiff base metal (II) complex compound carried out the general molecular structure is proposed as in Fig. 7.
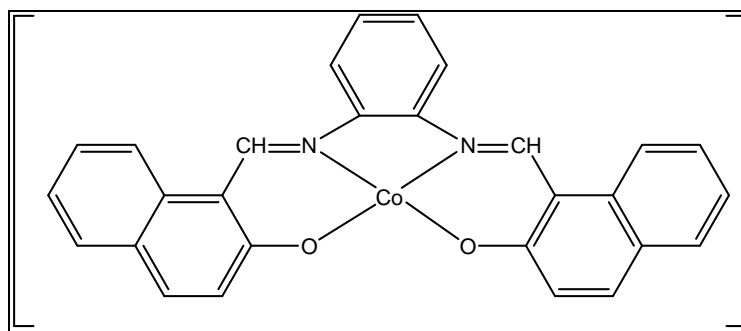

Figure 7: Proposed molecular structure of complex

5. References

i. Bhattacharjee, C. R. Das, G. and Mondal, P. (2011). "Photoluminescent Hemidisc-Shaped liquid Crystalline Nickel (II) Schiff-Base Complexes" Eur. *J. Inorg. Chem.,* 5390–5396.

ii. Bowmaker, G. A., Chaichit, N., Pakawatchai, C., Skelton B. W., and White, A. H., (2008) "Solvent-assisted mechanochemical synthesis of metal complexes" *Dalton Transactions*, 2926-2928.

iii. Chieng, N., Rades T. and Aaltonen, J. (2011): "An overview of recent studies on the analysis of pharmaceutical polymorphs" *Journal of Pharmaceutical and Biomedical Analysis*, 55, 618-644.

iv. Dharamraj N. Viswanathanmurthi P. and Natarajan K. (2001) "Ruthenium (II) complexes containing bidentate Schiff bases and their antifungal activity" Transition Metal Chemistry, 26(1):105-109

v. Fernandez-Bertran, J. and Reguera, E. (1997); "Mechanochemical reaction in alkali halide pressed disks" *Solid State Ionics*, **93**, 139-146.

vi. Friscic, T. (2010): "New opportunities for materials synthesis using mechanochemistry" *Journal of Materials Chemistry*, **20**, 7599-7605.

vii. Friscic, T. (2012): "Supramolecular concepts and new techniques in mechanochemistry: cocrystals, cages, rotaxanes, open metal–organic frameworks" *Chemical Society Reviews*, 41, 3493-3510.

viii. Friscic, T. and Jones, W. (2009): "Recent advances in understanding the mechanism of Cocrystals formation via grinding" *Crystal Growth & Design*, 9, 1621-1637.

ix. Germain, M. E. and Knapp, M. J. (2008). "Turn-on fluorescence detection of H_2O_2 and TATP" *Inorg. Chem.,* 47, 9748–9750

x. Idage, B. B. Idage, S. B. Kasegaonkar, A. S. and Jadhav, R. V. (2010). "Ring opening polymerization of dilactide using salen complexes as catalyst". *Mater. Sci. Eng., B*, 168, 193–198.

xi. Jacobsen, E. N., Zhang, W., Muci, A. R., Ecker, J. R. and Deng, L. J. (1991). "Highly enantioselective epoxidation catalysts derived from 1,2-diaminocyclohexane". *Am. Chem. Soc.,* 113, 7063–7064

xii. James, S. L., Adams, C. J., Bolm, C., Braga, D. Collier, P., Friscic, T. Grepioni, F., Harris, K. D. M., Hyett, G., Jones, W., A. Krebs, A., Mack, J., Maini, L., Orpen, A. G., Parkin, I. P., Shearouse, W. C., Steed, J. W. and Waddell, D. C. (2012): "Mechanochemistry: opportunities for new and cleaner synthesis" *Chemical Society Reviews*, 41, 413-447.

xiii. Kralova K. Kissova K. Svajlenova O. and Vanco J. (2000). "Biological Activity of Copper (II) N-Salicylideneaminoacidato Complexes." *Chem.* 58(5):357-361

xiv. Mason, B. P., Price, K. E., Steinbacher, J. L., Bogdan, A. R. and McQuade, D. T. (2007): "Greener Approaches to Organic Synthesis Using Microreactor Technology" *Chemical Reviews*, 107, 2300-2318

xv. Parekh J. Inamdhar P. Nair R. Baluja S. and Chanda S. (2005): "Synthesis and antibacterial activity of some Schiff bases derived from 4-aminobenzoic acid" *J. Serb Chem. Soc.* 70(10):1155-1161.

xvi. Ramon N., Mutijuraj V., Rovichandran S. and kulandaisamy A. (2003): "Synthesis, Characterization and Electrochemical Behavior of Cu(II), Co(II), Ni(II) and complexs derived from acetylacetone and panisidine and their antimicrobial activity", *Journal of Chemical Science,* 115(3):161-167.

xvii. Shan, N., Toda, F. and Jones, W. (2002): "Mechanochemistry and co-crystal formation: effect of solvent on reaction kinetics" *Chemical Communications*, 2372-2373.

xviii. Tweedy B. G. (1964). "Plant extracts with metal ions as potential antimicrobial agents" *Phyto Pathology* 55: 910

xix. Vaghasia Y. Nair R. Soni M. Bulaja S. Chanda S. (2004) "Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine" *J. Serb. Chem. Soc.* 69(12): 991-998.

xx. Yeamin R. Belayet H. and Saidul Islam M. (2003): "Actimicrobial Studies of Mixed Ligand Transition Metal Complexes of Phthailc acid and heterocyclic amine Bases" *Pakistan J. Biological sciences*, 6(17):1494-1496.